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COMPOSITIONS AND METHODS FOR THE TREATMENT OF NATURAL KILLER CELL RELATED DISEASES

Field of the Invention

The present invention relates to compositions and methods useful for the diagnosis and treatment of immune related diseases.

Background of the Invention

Immune related and inflammatory diseases are the manifestation or consequence of fairly complex, often multiple interconnected biological pathways which in normal physiology are critical to respond to insult or injury, initiate repair from insult or injury, and mount innate and acquired defense against foreign organisms. Disease or pathology occurs when these normal physiological pathways cause additional insult or injury either as directly related to the intensity of the response, as a consequence of abnormal regulation or excessive stimulation, as a reaction to self, or as a combination of these.

Though the genesis of these diseases often involves multistep pathways and often multiple different biological systems/pathways, intervention at critical points in one or more of these pathways can have an ameliorative or therapeutic effect. Therapeutic intervention can occur by either antagonism of a detrimental process/pathway or stimulation of a beneficial process/pathway.

Many immune related diseases are known and have been extensively studied. Such diseases include immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, *etc.*

Immune related diseases could be treated by suppressing the immune response. Using neutralizing antibodies that inhibit molecules having immune stimulatory activity would be beneficial in the treatment of immune-mediated and inflammatory diseases. Molecules which inhibit the immune response can be utilized (proteins directly or via the use of antibody agonists) to inhibit the immune response and thus ameliorate immune related disease.

Natural killer (NK) cells are an important effector cell of the innate immune system. They are specialized to effect killing against host cells that have either been infected by viruses, parasites or that have become cancerous. Phenotypically, NK cells are large granular lymphocytes that constitute ~2 % of the circulating lymphocyte population. They are commonly identified by cell surface expression of CD56 and CD16. NK cells mature in the bone marrow from a CD34+ precursor cell that they share with T cells. The mature NK cell, shares expression of CD8, cytolytic machinery, and some KIRs, with T cells, but remains distinct from T cells by the lack of CD3 and the T cell receptors. Like cytotoxic T cells, they contain granules filled with pore forming protein, cytotoxins, serine esterases and proteoglycans that mediate lysis of target cells. Both cytotoxic T cells and NK cells kill on contact by binding to their targets and delivering their lethal burst of chemicals that produces holes in the target cell's membrane. Unlike cytotoxic T cells, NK cells do not need to recognize a specific antigen before initiating lysis. Rather, NK cell activation can be mediated by growth factors and cytokines such as, IL-2, IL-12 and IL-15 have been shown to mediate

proliferative and cytotoxic activities or by a delicate balance between two classes of NK cell receptors, one that activates the cells, and another that inhibits. Killer Ig-like receptors (KIRs) are NK cell receptors that transmit an inhibitory signal if they encounter class I MHC molecules on a cell surface. This is important for killing of both cancerous cells and virally infected cells. Because viruses often suppress class I MHC expression in cells they infect, the virus-infected cell becomes susceptible to killing by NK cells. Likewise, cancer cells have reduced or no class I MHC expression also become susceptible to killing by NK cells. Natural cytotoxicity receptors (NCRs) constitute a family of activating receptors on NK cells. In some effector-target systems, the surface density of NCRs correlates with the cytolytic activity of the NK cells, while in other systems killing requires cooperation between NCR, another activating receptor NKG2D and its adaptor polypeptide DAP10. Additionally, the strength of the stimulatory signals can be influenced by engagement of co-receptors such as 2B4 and NTB-A. The ligands for NCRs and NKG2D, hemoglutanins and MICA, MICB respectively are not expressed by most normal cells, but are induced in most tumor cell lines. Expression of the ligands by tumor cells triggers a dramatic immune response resulting in tumor cell rejection.

Activation of NK cells with IL-15 or IL-12 have been shown to induce both cytotoxic and proliferative effects. Junctional adhesion molecule 2 (JAM2) has been shown to bind to NK cells and has been hypothesized to play a role in lymphocyte extravasation to sites of inflammation. Therefore, a DNA microarray experiment comparing differential expression of genes from these three modes of activation versus resting NK cells has the potential to reveal novel genes or novel gene associations with NK cell activity. Therapeutic antibodies, peptides or small molecules could be developed to target specific genes revealed by these microarrays for the treatment of immune mediated inflammatory diseases and malignancies.

Despite the above research in NK cells, there is a great need for additional diagnostic and therapeutic agents capable of detecting the presence of NK cell mediated disorders in a mammal and for effectively reducing these disorders. Accordingly, it is an objective of the present invention to identify polypeptides that are differentially expressed in activated NK cells as compared to resting NK cells, and to use those polypeptides, and their encoding nucleic acids, to produce compositions of matter useful in the therapeutic treatment and diagnostic detection of NK cell mediated disorders in mammals.

Summary of the Invention

A. Embodiments

The present invention concerns compositions and methods useful for the diagnosis and treatment of immune related disease in mammals, including humans. The present invention is based on the identification of proteins (including agonist and antagonist antibodies) which are a result of stimulation of the immune response in mammals. Immune related diseases can be treated by suppressing or enhancing the immune response. Molecules that enhance the immune response stimulate or potentiate the immune response to an antigen. Molecules which stimulate the immune response can be used therapeutically where enhancement of the immune response would be beneficial. Alternatively, molecules that suppress the immune response attenuate or reduce the immune response to an antigen (*e.g.*, neutralizing antibodies) can be used therapeutically where attenuation of the immune response would be beneficial (*e.g.*, inflammation).

Accordingly, the PRO polypeptides, agonists and antagonists thereof are also useful to prepare medicines and medicaments for the treatment of immune-related and inflammatory diseases. In a specific aspect, such medicines and medicaments comprise a therapeutically effective amount of a PRO polypeptide, agonist or antagonist thereof with a pharmaceutically acceptable carrier. Preferably, the admixture is sterile.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprises contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native sequence PRO polypeptide. In a specific aspect, the PRO agonist or antagonist is an anti-PRO antibody.

In another embodiment, the invention concerns a composition of matter comprising a PRO polypeptide or an agonist or antagonist antibody which binds the polypeptide in admixture with a carrier or excipient. In one aspect, the composition comprises a therapeutically effective amount of the polypeptide or antibody. In another aspect, when the composition comprises an immune stimulating molecule, the composition is useful for: (a) increasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) stimulating or enhancing an immune response in a mammal in need thereof, (c) increasing the proliferation of NK cells in a mammal in need thereof in response to an antigen, (d) stimulating the activity of NK cells or (e) increasing the vascular permeability. In a further aspect, when the composition comprises an immune inhibiting molecule, the composition is useful for: (a) decreasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) inhibiting or reducing an immune response in a mammal in need thereof, (c) decreasing the activity of NK cells or (d) decreasing the proliferation of NK cells in a mammal in need thereof in response to an antigen. In another aspect, the composition comprises a further active ingredient, which may, for example, be a further antibody or a cytotoxic or chemotherapeutic agent. Preferably, the composition is sterile.

In another embodiment, the invention concerns a method of treating an immune related disorder in a mammal in need thereof, comprising administering to the mammal an effective amount of a PRO polypeptide, an agonist thereof, or an antagonist thereto. In a preferred aspect, the immune related disorder is selected from the group consisting of: systemic lupus erythematosis, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis, idiopathic inflammatory myopathies, Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious, autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft-versus-host-disease.

In another embodiment, the invention provides an antibody which specifically binds to any of the

above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody. In one aspect, the present invention concerns an isolated antibody which binds a PRO polypeptide. In another aspect, the antibody mimics the activity of a PRO polypeptide (an agonist antibody) or conversely the antibody inhibits or neutralizes the activity of a PRO polypeptide (an antagonist antibody). In another aspect, the antibody is a monoclonal antibody, which preferably has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a monoclonal antibody, a single-chain antibody, or an anti-idiotypic antibody.

In yet another embodiment, the present invention provides a composition comprising an anti-PRO antibody in admixture with a pharmaceutically acceptable carrier. In one aspect, the composition comprises a therapeutically effective amount of the antibody. Preferably, the composition is sterile. The composition may be administered in the form of a liquid pharmaceutical formulation, which may be preserved to achieve extended storage stability. Alternatively, the antibody is a monoclonal antibody, an antibody fragment, a humanized antibody, or a single-chain antibody.

In a further embodiment, the invention concerns an article of manufacture, comprising:

(a) a composition of matter comprising a PRO polypeptide or agonist or antagonist thereof;

(b) a container containing said composition; and

(c) a label affixed to said container, or a package insert included in said container referring to the use of said PRO polypeptide or agonist or antagonist thereof in the treatment of an immune related disease. The composition may comprise a therapeutically effective amount of the PRO polypeptide or the agonist or antagonist thereof.

In yet another embodiment, the present invention concerns a method of diagnosing an immune related disease in a mammal, comprising detecting the level of expression of a gene encoding a PRO polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample as compared to the control sample indicates the presence of immune related disease in the mammal from which the test tissue cells were obtained.

In another embodiment, the present invention concerns a method of diagnosing an immune disease in a mammal, comprising (a) contacting an anti-PRO antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the antibody and a PRO polypeptide, in the test sample; wherein the formation of said complex is indicative of the presence or absence of said disease. The detection may be qualitative or quantitative, and may be performed in comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. A larger quantity of complexes formed in the test sample indicates the presence or absence of an immune disease in the mammal from which the test tissue cells were obtained. The antibody preferably carries a detectable label. Complex formation can be monitored, for example, by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. The test sample is usually obtained from an individual suspected of having a deficiency or abnormality of the immune system.

In another embodiment, the invention provides a method for determining the presence of a PRO